REMARKS

Rejections under 35 U.S.C. §101

All claims have been rejected under 35 U.S.C. § 101 as reading on nonstatutory subject matter. Applicants have limited the claims by amendment herein to methods of making transgenic **non-human** mammals, as per the Examiner's suggestion, and respectfully submit that the rejection has been obviated by amendment and should be withdrawn.

Rejections under 35 U.S.C. §112, ¶ 1, Scope of Enablement

The Examiner rejects all pending claims under 35 U.S.C. § 112, first paragraph, in three separate scope of enablement rejections. Applicants respectfully traverse.

Scope of "phenotype"

Noting that "[t]he claimed invention is drawn to methods of making transgenic mammals with no particular phenotype," and that applicants' specification similarly "discloses no phenotype for the genetically modified mammals," the Examiner concludes that "undue experimentation would have been required for one of skill in the art to make and use the claimed invention."

The Examiner's observations are correct: applicants' claimed methods are generic to, and thus read on, any user-desired phenotype, and the methods taught in applicants' specification are of commensurate scope.

The conclusion that the Examiner draws from these observations, however, is incorrect, and manifestly so.

Were it otherwise, were it always necessary to claim methods of transgenesis in association with a "particular phenotype", the following claims would perforce equally be said to be invalid under 35 U.S.C. § 112, first paragraph:

A method of producing a transgenic bovine, the method comprising:

obtaining an ovum from bovine ovaries;

maturing the ovum in vitro;

fertilizing the mature ovum in vitro to form a zygote;

introducing a transgene into the zygote in vitro;

maturing the zygote to a preimplantation stage embryo in vitro; and

transplanting the embryo into a recipient female bovine, wherein the female bovine gestates the embryo to produce a transgenic bovine.[1]

A method for regenerating a fertile transformed wheat plant to contain foreign DNA comprising the steps of:

(a) producing regenerable tissue from said plant to be transformed:

(b) transforming said regenerable tissue with said foreign DNA where said foreign DNA comprises a selectable DNA sequence, where said sequence functions in a regenerable tissue as a selection device;

(c) between about one day to about 21 days after step b) placing said regenerable tissue from step b) in a medium capable of producing shoots from said tissue wherein said medium further contains a compound used to select regenerable tissue containing said selectable DNA sequence to allow identification or selection of the transformed regenerated tissue;

(e) after at least one shoot has formed from the selected tissue of step c), transferring said shoot to a second medium capable of producing roots from said shoot to produce a plantlet; and,

(e) growing said plantlet into a fertile, transgenic wheat plant wherein the foreign DNA is transmitted to progeny plants in mendelian fashion.[2]

A method for producing a transgenic pig comprising:

a) obtaining a porcine embryo comprising at least three blastomeres;

b) introducing at least one clone of isolated nucleic acid molecules into at least one blastomere of the embryo;

c) transferring the embryo to a surrogate female pig;

d) developing the embryo into at least the fetal stage; and

e) developing the fetus into a transgenic pig.[3]

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[1] Claim 1, U.S. Pat. No. 5,633,076.

[2] Claim 1, U.S. Pat. No. 5,631,152.

A method of genetically engineering a conifer, the method comprising the steps of

placing immature embryos of the conifer on a target surface;

physically accelerating at the embryos carrier particles which are much smaller than the cells of the embryos, the carrier particles carrying copies of a foreign genetic construction including at least one foreign gene of interest and one selectable marker gene;

inducing the embryos to form proliferative callus which is capable of forming somatic embryos;

during the step of inducing, culturing the callus in a medium containing a selection agent for which the selectable marker gene confers resistance so as to select for embryogenic callus which is totally transformed and which expresses the gene of interest;

inducing somatic embryos to form from the callus; and

regenerating the somatic embryos thus produced into clonal transgenic conifer plants.[4]

A method of producing a transgenic mouse having a genome comprising a modification of a target DNA sequence, said method comprising:

(a) transforming a population of mouse embryonic stem cells with a PNS vector;

(b) identifying a cell having said genome by selecting for cells containing said positive selection marker and against cells containing said negative selection marker and analyzing DNA from cells surviving selection for the presence of said modification;

(c) inserting said cell into a mouse embryo;

(d) propagating a mouse from said embryo; and

(e) breeding said mouse to produce said transgenic mouse having said genome;

wherein said PNS vector comprises:

(1) a first homologous vector DNA sequence capable of homologous recombination with a first region of said target DNA sequence,

(2) a positive selection marker DNA sequence capable of conferring a positive selection characteristic in said cells,

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[3] Claim 1, U.S. Pat. No. 6,498,285.

[4] Claim 1, U.S. Pat. No. 5,681,730.

(3) a second homologous vector DNA sequence capable of homologous recombination with a second region of said target DNA sequence, and

(4) a negative selection marker DNA sequence, capable of conferring a negative selection characteristic in said cells, but substantially incapable of homologous recombination with said target DNA sequence,

wherein the spatial order of said sequences in said PNS vector is: said first homologous vector DNA sequence, said positive selection marker DNA sequence, said second homologous vector DNA sequence and said negative selection marker DNA sequence as shown in FIG. 1,

wherein the 5'-3' orientation of said first homologous vector sequence relative to said second homologous vector sequence is the same as the 5'-3' orientation of said first region relative to said second region of said target sequence;

wherein the vector is capable of modifying said target DNA sequence by homologous recombination of said first homologous vector DNA sequence with said first region of said target sequence and of said second homologous vector DNA sequence with said second region of said target sequence.[5]

A method of transforming a corn plant cell or plant tissue using an Agrobacterium mediated process comprising the steps of:

inoculating a transformable plant cell or tissue from a corn plant with Agrobacterium containing at least one genetic component capable of being transferred to the plant cell or tissue in an inoculation media containing an effective amount of at least one antibiotic that inhibits or suppresses the growth of Agrobacterium;

co-culturing the transformable plant cell or tissue after the inoculating step in a medium capable of supporting growth of plant cells or tissue expressing the genetic component, said medium not containing said antibiotic;

selecting transformed plant cells or tissue; and

regenerating a transformed corn plant expressing the genetic component from the selected transformed plant cells or tissue.[6]

A method for the production of fertile, transgenic plants wherein the transgenic plant has a DNA sequence of interest integrated at a

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[5] Claim 1, U.S. Pat. No. 5,627,059.

[6] Claim 1, U.S. Pat. No. 6,603,061.

predetermined DNA sequence of the plant, said method comprising the steps of

introducing into plant cells a DNA construct comprising

a multifunctional DNA sequence flanked by a pair of directly repeated site-specific recombination sequences,

said multifunctional DNA sequence comprising a gene encoding a selectable marker, and a DNA sequence of interest,

wherein said DNA sequence of interest is flanked by nucleotide sequences sharing homology to the predetermined nucleotide sequence present in the plant cell, and the selectable marker gene is operably linked to regulatory sequences capable of expressing the gene in the plant cell,

selecting for plant cells having said DNA construct integrated into the DNA of the plant cell,

eliminating randomly inserted DNA constructs through expression of a recombinase gene capable of initiating recombination at the site-specific recombinase sequences in the plant cells,

identifying cells having said DNA sequence of interest integrated into the plant's DNA via a homologous recombination event, and

culturing said identified cells to generate an entire plant.[7]

A method for producing transgenic wheat plants, comprising the steps:

(a) obtaining a Type C embryogenic wheat callus;

(b) delivering heterologous DNA into the cells of said callus by bombarding said callus with accelerated microprojectiles adsorbed with said DNA;

(c) selecting for and growing transgenic wheat cells; and

(d) regenerating transgenic wheat plants from said transgenic wheat cells.[8]

A method of making a non-human mammal harboring a biologically functional non-native cell comprising:

(i) selectively destroying the endogenous germ cell population in the seminiferous tubules of a first non-human mammal, leaving intact the supporting cells comprising Sertoli cells in said tubules; and

(ii) colonizing said seminiferous tubules of said first non-human mammal with spermatogonia from a second non-human mammal of the same species as the first non-human mammal, wherein said colonizing

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[7] Claim 1, U.S. Pat. No. 5,527,695.

[8] Claim 1, U.S. Pat. No. 5,405,765.

comprises injecting a solution containing said spermatogonia from said second mammal into said seminiferous tubules or into the lumen of the rete testes into the efferent duct leading into the epididymis of said first mammal,

wherein following colonization, said spermatogonia from second mammal produce spermatozoa which are capable of repopulating said seminiferous tubules, fertilizing ova, and producing viable offspring.[9]

Were it always necessary to claim methods of transgenesis in association with a "particular phenotype", all of those claims would have to be said to be invalid under 35 U.S.C. § 112, first paragraph. And they cannot: each is statutorily presumed to be enabled across the entirety of its scope, 35 U.S.C. § 282; 35 U.S.C. § 112, ¶ 1, and the Examiner is prohibited from adducing any argument that would cast the validity of such claims in doubt, M.P.E.P. § 1701.

And that is of course the correct result: it should be no more necessary to limit claims drawn to improved methods of transgenesis to a particular transgenic phenotype than it is necessary to limit claims drawn to improved methods of nucleic acid sequencing to a particular nucleic acid to be sequenced, or to limit claims drawn to improved methods of propagating hybridomas to a particular specificity of secreted antibody, or to limit claims drawn to improved methods of weighing solids to a particular type of solid to be weighed. Indeed, nothing in the first paragraph of section 112 mandates that the genotypic alteration effected by applicants' claimed methods change the transgenic animal's immediately observable phenotype *at all*.[10]

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[9] Claim 1, U.S. Pat. No. 5,858,354.

[10] The Examiner's echo of *Brenner v. Manson*, that "a method of making a product has use only if the product made has use," is best answered thus: the methods here claimed, and the products thereof, have all of the uses, respectively, as do the transgenesis methods of earlier-issued claims that are similarly broad to phenotype.

Imagine claim 32 recast in Jepson format: "A method of producing transgenic non-human mammals, the improvement comprising: introducing into at least one mammalian zygote at least

The question is not whether applicants are permitted to claim improved methods that are broad to any desired phenotype -- indeed, sufficiently broad to read on a transgenic animal with a wild-type phenotype -- but, rather, whether applicants' specification, coupled with information known in the art, is sufficient to enable applicants' methods across a sufficient number of genetic (not *phenotypic*) alterations to meet the requirements of 35 U.S.C. § 112, first paragraph.

Applicants respectfully submit that it does, and that the Examiner has adduced neither evidence nor reasoned argument to suggest otherwise. With respect, the Examiner has failed to establish a *prima facie* case of inadequate scope of enablement, the rejection is in error, and the rejection should be withdrawn.

Scope of "genotype"

The Examiner rejects claims 32 - 37 and 41 - 70 on grounds that, "[w]hile the specification enables single base substitutions, no guidance is provided for making other types of changes to the genome" such as "inserting entire genes into specifically selected sites of the target genome."

Applicants respectfully disagree: the specification clearly discloses that the methods of the present invention may be used, *inter alia*, to effect just such insertions. By way of nonlimiting example,

> In general, transgenic animals are made with any number of changes. Exogenous sequences, or extra copies of endogenous sequences, including structural genes and regulatory sequences, may be added to the animal, as outlined below. Endogenous sequences (again, either genes or regulatory sequences) may be disrupted, i.e. via insertion, deletion or substitution, to prevent expression of endogenous proteins. Alternatively, endogenous sequences may be modified to alter their biological function,

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one recombinase and at least two single-stranded targeting polynucleotides . . . . " Unless the introduction of recombinase in concert with the targeting polynucleotides is less efficacious than introduction of the transgene alone, the method of claim 32 can be no less useful than a method, lacking use of a recombinase, that is earlier-claimed.

for example via mutation of the endogenous sequence by insertion, deletion or substitution.

Accordingly, [t]he methods of the present invention are useful to add exogenous DNA sequences, such as exogenous genes or regulatory sequences, extra copies of endogenous genes or regulatory sequences, or exogenous genes or regulatory sequences, to a transgenic plant or animal. This may be done for a number of reasons: for example, adding one or more copies of a wild-type gene can increase the production of a desirable gene product; adding or deleting one or more copies of a therapeutic gene can alleviate a disease state, or to create an animal model of disease. Adding one or more copies of a modified wild type gene may be done for the same reasons. Adding therapeutic genes or proteins may yield superior transgenic animals, for example for the production of therapeutic or nutriceutical proteins. Adding human genes to non-human mammals may facilitate production of human proteins and adding regulatory sequences derived from human or non-human mammals may be useful to increase or decrease the expression of endogenous or exogenous genes. Such inserted genes may be under the control of endogenous or exogenous regulatory sequences, as described herein.[11]

In a preferred embodiment, an insertion sequence comprises a gene which not only disrupts the endogenous gene, thus preventing its expression, but also can result in the expression of a new gene product. Thus, in a preferred embodiment, the disruption of an endogenous gene by an insertion sequence gene is done in such a manner to allow the transcription and translation of the insertion gene. An insertion sequence that encodes a gene may range from about 50 bp to 5000 bp of cDNA or about 5000 bp to 50000 bp of genomic DNA. As will be appreciated by those in the art, this can be done in a variety of ways. In a preferred embodiment, the insertion gene is targeted to the endogenous gene in such a manner as to utilize endogenous regulatory sequences, including promoters, enhancers or a regulatory sequence. In an alternate embodiment, the insertion sequence gene includes its own regulatory sequences, such as a promoter, enhancer or other regulatory sequence etc.

Particularly preferred insertion sequence genes include, but are not limited to, genes which encode therapeutic and nutriceutical proteins, and reporter genes. Suitable insertion sequence genes which may be inserted into endogenous genes include, but are not limited to, nucleic acids which encode those genes listed as suitable endogenous genes for alterations, above, particularly mammalian enzymes, mammalian antibodies, mammalian proteins including serum albumin as well as mammalian therapeutic genes. In a preferred embodiment, the inserted mammalian

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[11] Specification, p. 45, lines 8 - 29.

gene is a human gene. Suitable reporter genes are those genes which encode detectable proteins, such as the genes encoding luciferase, .beta.-galactosidase (both of which require the addition of reporter substrates), and the fluorescent proteins, including green fluorescent protein (GFP), blue fluorescent protein (BFP), yellow fluorescent protein (YFP), and red fluorescent protein (RFP).

Thus, in a preferred embodiment, the targeted sequence modification creates a sequence that has a biological activity or encodes a polypeptide having a biological activity. In a preferred embodiment, the polypeptide is an enzyme with enzymatic activity. In another preferred embodiment, the polypeptide is an antibody. In a third preferred embodiment, the polypeptide is a structural protein.

In addition, the insertion sequence genes may be modified or variant genes, i.e. they contain a mutation from the wild-type sequence. Thus, for example, modified genes including, but not limited to, improved therapeutic genes, modified (.alpha.-lactalbumin genes that do not encode any phenylalanine residues, or human enzyme or human antibody genes that do not encode any phenylalanine residues.[12]

Typically, a targeting polynucleotide (or complementary polynucleotide pair) has a portion or region having a sequence that is not present in the preselected endogenous targeted sequence(s) (i.e., a nonhomologous portion or mismatch) which may be as small as a single mismatched nucleotide, several mismatches, or may span up to about several kilobases or more of nonhomologous sequence. Generally, such nonhomologous portions are flanked on each side by homology clamps, although a single flanking homology clamp may be used. Nonhomologous portions are used to make insertions, deletions, and/or replacements in a predetermined endogenous targeted DNA sequence, and/or to make single or multiple nucleotide substitutions in a predetermined endogenous target DNA sequence so that the resultant recombined sequence (i.e., a targeted recombinant endogenous sequence) incorporates some or all of the sequence information of the nonhomologous portion of the targeting polynucleotide(s). Thus, the nonhomologous regions are used to make variant sequences, i.e. targeted sequence modifications. Additions and deletions may be as small as 1 nucleotide or may range up to about 2 to 4 kilobases or more. In this way, site directed modifications may be done in a variety of systems for a variety of purposes.[13]

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[12] Specification, p. 47, line 9 - p. 48, line 12.

[13] Specification, p. 53, lines 15 - 30.

It is beyond debate that applicants' specification clearly and unambiguously teaches that the methods of the present invention may be used to effect large insertions.

"[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 439 F.2d 220, 223 (C.C.P.A. 1971) (emphasis added); quoted with approval in *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995).

To shoulder that burden, the Examiner notes comments in applicants' ***own specification*** that "as the region of heterology increases, the stability of four-strand hybrids decreases significantly," and concludes from that observation that "the efficiency of recombination would be expected to be much lower for larger deletions or insertions." Office Action at 3.

Logically, applicants' specification, read as a whole, must be understood to assert that, *notwithstanding* any decrease in stability of four-strand hybrids incident to increases in heterology, large insertions are nonetheless possible. The Examiner has adduced neither reason nor evidence sufficient to cast doubt on the objective truth of such statement.

Solely to expedite prosecution, admitting neither to the sufficiency of the Examiner's *prima facie* case of inadequate scope of enablement nor to an attendant shift of the burden of production to applicants, applicants respectfully invite the Examiner's attention to Maga *et al.*, "Increased efficiency of transgenic livestock production," *Transgenic Research* 12:485-496 (2003), attached hereto as Exhibit A. As reported, the use of RecA protein results in significant increase in transgene integration frequencies. The claimed methods work. They are fully enabled. The rejection is in error and should be withdrawn.

Scope of mammalian species

The Examiner rejects all pending claims under 35 U.S.C. § 112, first paragraph, on the ground that the specification fails to provide an enabling disclosure for making *targeted* changes to any mammal other than the mouse.

The USPTO has already established as fully enabled applicants' methods for targeting and altering, by homologous recombination, a pre-selected target DNA sequence in ***any eukaryotic cell*** to make a targeted sequence modification, the method comprising introducing into at least one eukaryotic cell at least one recA recombinase and at least two single-stranded targeting polynucleotides which are substantially complementary to each other, and which further comprise a homology clamp that substantially corresponds to or is substantially complementary to a preselected target DNA sequence. Claim 1, U.S. Pat. No. 5,763,240 (to which the instant application claims priority); 35 U.S.C. § 282; 35 U.S.C. § 112, first paragraph.

The rejection is in error and should be withdrawn.

Rejections under 35 U.S.C. § 112, ¶ 2

Claims 32 - 37 and 41 - 70 are rejected as indefinite under 35 U.S.C. § 112, ¶ 2, on grounds that the method steps recited in the body of the claims do not result in the transgenic mammal of the preamble. The rejection has been obviated by amendment of claim 32. No new matter has been added; support for the term "fostering" can be found in the specification at page 95, line 12.

Claims 32 - 37 and 41 - 70 are rejected as indefinite for recitation of modified endogenous nucleic acid", "because it is unclear relative to what standard or point of reference the endogenous nucleic acid is considered to be 'modified'", and because "it is unclear what would be regarded as an 'endogenous nucleic acid.'"

Applicants traverse the rejection: the terms are standard in the art and would be clearly understood by one skilled in that art.[14] Applicants respectfully request reconsideration.

Claims 34 and 65 are rejected as indefinite for recitation of "farm" mammal. The rejection has been obviated: claim 34 has been amended to recite the Markush group of livestock previously presented in claim 35, with cancellation of claim 35 and consequential amendment of claim 65.

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[14] Note, for example, claim 1 of U.S. Pat. No. 6,673,986:

1. A transgenic mouse comprising in its germline a modified genome wherein said modification comprises inactivated endogenous immunoglobulin heavy chain loci in which all of the J segment genes from both copies of the immunoglobulin heavy chain locus are deleted to prevent rearrangement and to prevent formation of a transcript of a rearranged locus and the expression of an endogenous immunoglobulin heavy chain from the inactivated loci.

CONCLUSION

Applicants submit that the present application is in condition for allowance, and respectfully request the same.

Respectfully submitted,

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Attachment (Exhibit A): Maga et al., "Increased efficiency of transgenic livestock production," *Transgenic Research* 12:485-496 (2003),